Supplementary Figure Legends

Figure S1. Structural determination, Related to Figure 2.

(A) The repeat:antirepeat duplex in the sgRNA for crystallization. The internal loop in the crRNA repeat-tracrRNA antirepeat duplex was replaced with G:C base pairs for crystallization, and the crRNA and the tracrRNA were fused by a GAAA tetraloop.

(B) The $2mF_O - DF_C$ electron density map (contoured at 1.5σ). Water molecules are shown as red spheres.

Figure S2. Comparison of the overall structures of the Cas9 orthologs, Related to Figure 2.

(A–C) Overall structures of FnCas9 (A), SpCas9 (PDB: 4UN3) (B) and SaCas9 (PDB: 5AXW) (C) in complexes with the sgRNA and the target DNA. The HNH domains are omitted for clarity. The sgRNA in the SpCas9 quaternary complex (PDB: 4UN3) contains stem loops 1–2 and lacks stem loop 3, whereas the sgRNA in the SpCas9 ternary complex (PDB: 4UN3) contains stem loops 1–3. Thus, in (B), the sgRNA (PDB: 4OO8) is docked into the SpCas9–DNA complex (PDB: 4UN3).

Figure S3. Inter-domain interactions in FnCas9, Related to Figure 2.

(A) Interaction between the WED and REC1/REC2 domains.

(B) Interaction between the WED and REC1 domains.

(C) Interaction between the WED and PI domains.

(D) Interaction between the RuvC and REC3 domains.

Hydrogen-bonding interactions are shown as dashed lines.

Figure S4. Structure of the FnCas9 sgRNA scaffold, Related to Figure 3.

(A–C) Structures of the repeat:antirepeat duplex (A), stem loop 1 (B) and stem loop 2 (C) (stereo view). Hydrogen-bonding interactions are shown as dashed lines. In (B), C53 and U63 are depicted as semi-transparent space-filling models, highlighting the base-stacking interaction.

Figure S5. DNA targeting mechanism of FnCas9, Related to Figure 5.

(A) Recognition of the sgRNA seed region by the bridge helix (BH) and the REC1 domain (stereo view). The target DNA strand is omitted for clarity.

(B) Recognition of the +1 phosphate group by the phosphate lock loop (PLL) (stereo view). Hydrogen-bonding interactions are shown as dashed lines.

Figure S6. Recognition of sgRNA scaffolds by the Cas9 orthologs, Related to Figure 5.

(A–C) Recognition of the sgRNA scaffolds by FnCas9 (A), SpCas9 (B) and SaCas9 (C). R:AR duplex, repeat:antirepeat duplex.

(D–E) Recognition of the repeat:antirepeat duplex (D), stem loop 2 (E), and the SL1–SL2 linker (F) by FnCas9. In (F), the REC3 β-hairpin is inserted between stem loop 2 and the SL1–SL2 linker.

Figure S7. Recognition of the sgRNA core regions by the Cas9 orthologs, Related to Figure 5.

(A–C) Recognition of the sgRNA core regions by FnCas9 (A), SpCas9 (B) and SaCas9 (C) (stereo view). Hydrogen-bonding and electrostatic interactions are shown as dashed lines.

Table S1. Data Collection and Refinement Statistics, Related to Figure 2.

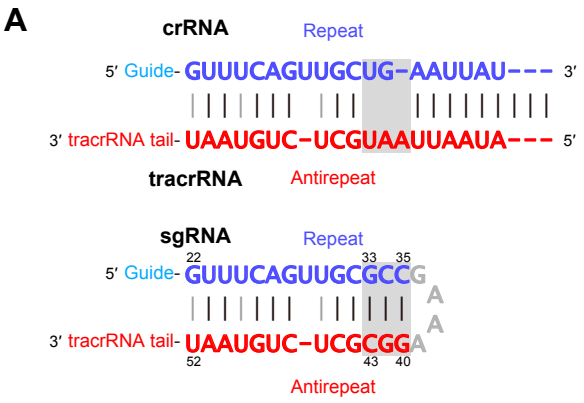
	TGG PAM (Native)	TGA PAM (Native)	TGG PAM (RHA variant)	TGG PAM (SeMet)
Data collection				
Beamline	SPring-8 BL41XU	SPring-8 BL41XU	SPring-8 BL41XU	SLS PXIII
Wavelength (Å)	1.0000	1.0000	1.0000	0.9780
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Cell dimensions				
a, *b*, *c* (Å)	81.9, 159.1, 96.8	81.6, 159.3, 96.7	81.6, 156.0, 96.7	81.3, 157.4, 96.5
α, *β*, *γ* (°)	90, 107.0, 90	90, 106.9, 90	90, 106.9, 90	90, 106.8, 90
Resolution (Å)*	46.3–1.7 (1.73–1.70)	46.3–1.7 (1.73–1.70)	46.3–1.7 (1.73–1.70)	19.9–2.3 (2.34–2.30)
R_{merge}	0.062 (0.72)	0.060 (1.42)	0.039 (0.77)	0.091 (1.02)
R_{pim}	0.037 (0.44)	0.024 (0.56)	0.024 (0.47)	0.026 (0.28)
$I/\sigma I$	14.2 (2.8)	19.6 (2.4)	20.3 (2.5)	19.1 (2.0)
Completeness (%)	96.3 (96.2)	98.8 (97.9)	98.9 (98.7)	99.7 (98.9)
Multiplicity	7.2 (7.2)	14.1 (14.3)	7.0 (7.1)	14.3 (13.8)
CC(1/2)	0.99 (0.65)	0.99 (0.86)	1.00 (0.74)	0.96 (0.86)
Refinement				
Resolution (Å)	46.3–1.7	49.7–1.7	46.3-1.7	
No. reflections	249,252	255,162	254,945	
R_{work}/ R_{free}	0.190 / 0.214	0.186 / 0.210	0.187 / 0.214	
No. atoms				
Protein	11,672	11,755	11,752	
Nucleic acid	2,786	2,786	2,786	
Ion	24	24	24	
Solvent	1,000	992	998	
B-factors (Å^2)				
Protein	50.3	50.2	52.1	
Nucleic acid	39.5	40.2	40.0	
Ion	52.7	54.2	53.4	
Solvent	34.7	40.3	40.6	
R.m.s. deviations				
Bond lengths (Å)	0.011	0.015	0.014	
Bond angles (°)	1.46	1.492	1.45	
Ramachandran plot (%)				
Favored region	97.4	97.2	96.9	
Allowed region	0.6	3.3	0.4	
Outlier region	0.1	0.1	0.1	

*Values in parentheses are for the highest resolution shell.

Table S2. Target Sequences of Mouse Zygotes, Related to Figure 7.

Gene	Target sequence	PAM	Indel detection
Tet1EX4	CAGGGAGCTCATGGAGACTAGG	TGA	*Bfa*I
Tet1EX4	CACTTGGTCCTGCCCCAAGGTG	TGT	*Eco*T14 I
Tet1EX4	GTGGCTGCTGTCAGGGAGCTCA	TGG	*Sac*I
Tet1EX4	ATGGAGACTAGGTGAGGAACTC	TGC	HMA

Click here to download Supplemental Figure s_figure1_160125.pdf



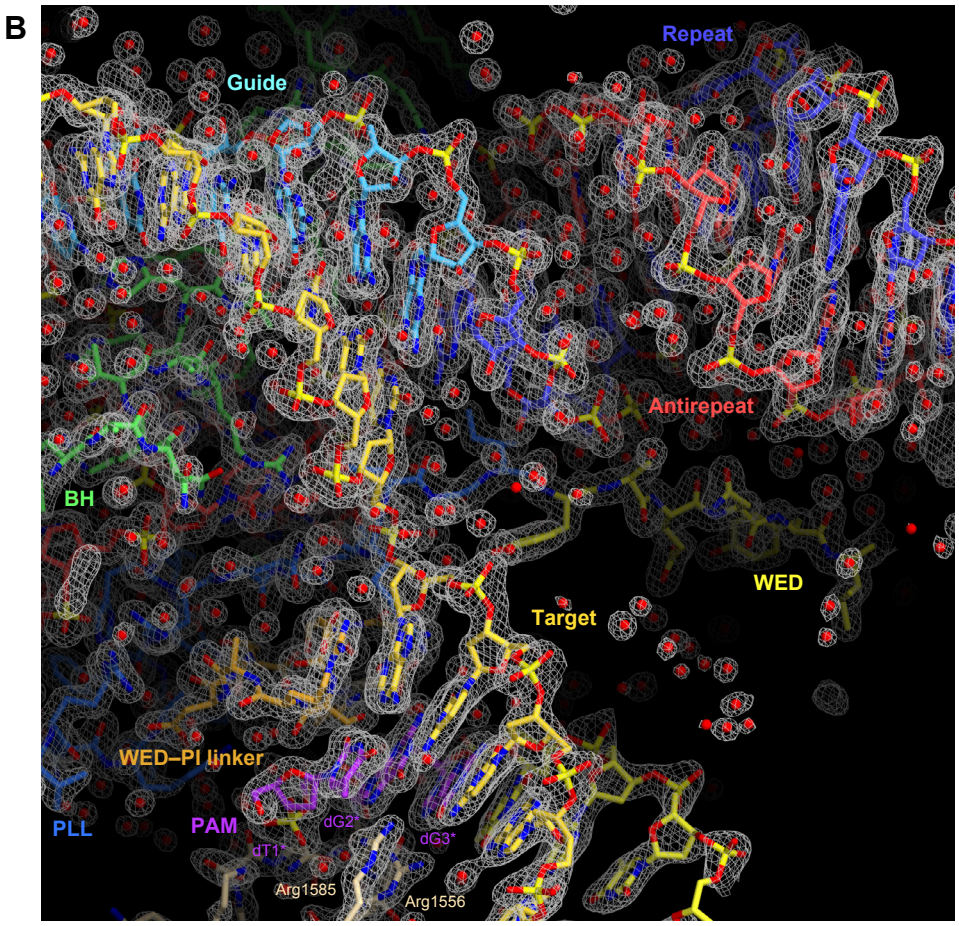


Figure S1

Click here to download Supplemental Figure s_figure2_160125.pdf

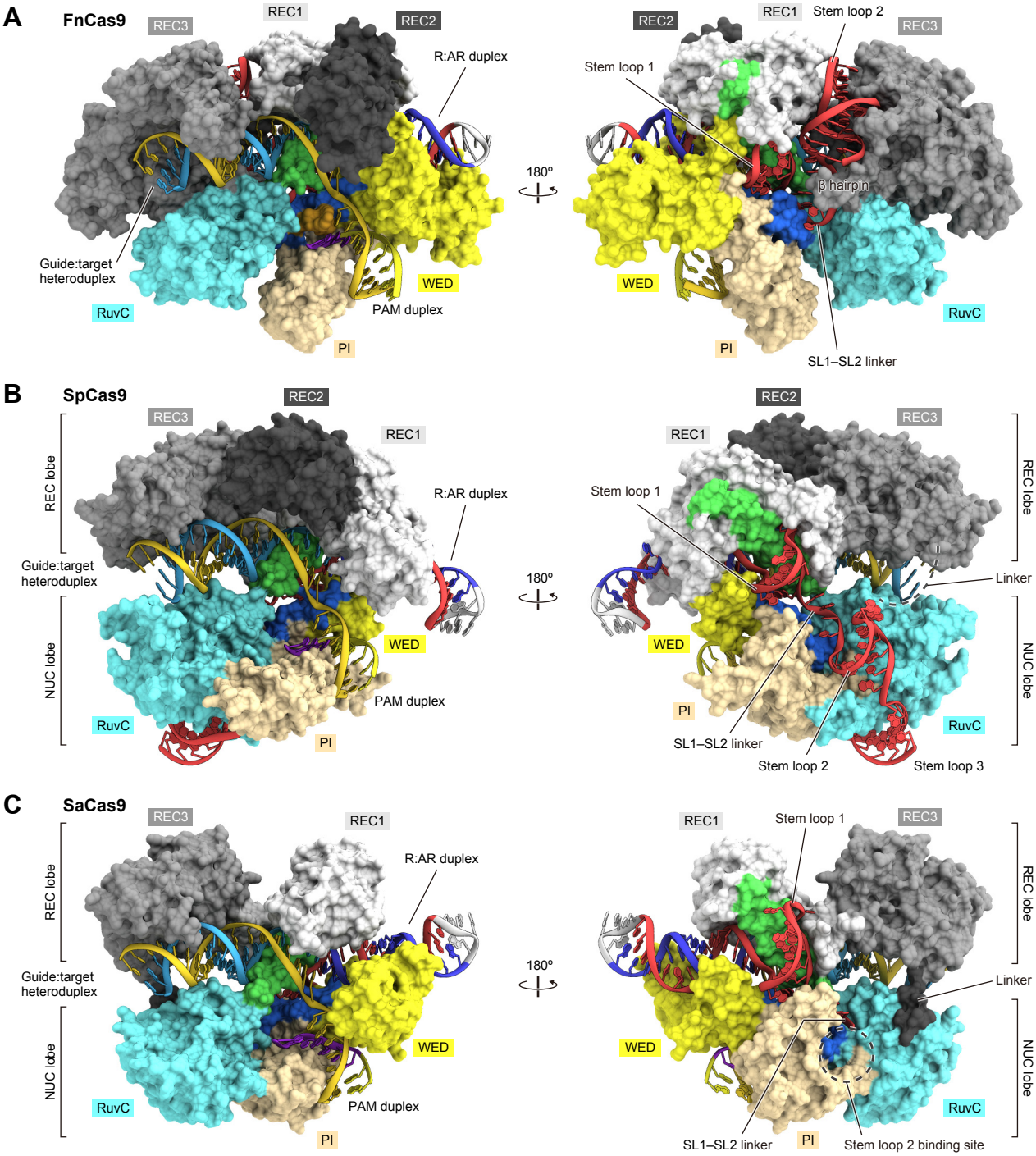


Figure S2

Click here to download Supplemental Figure s_figure3_160125.pdf

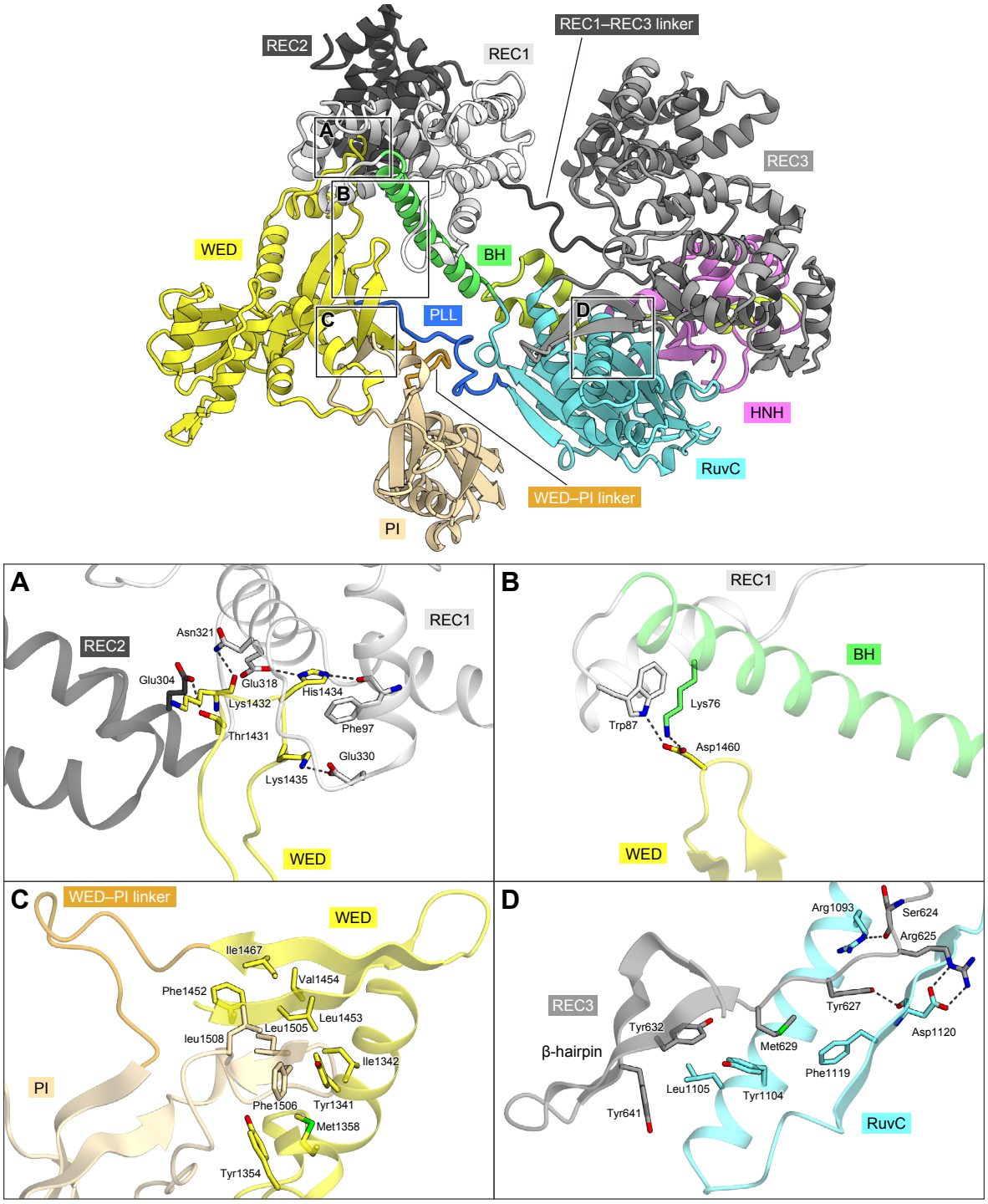


Figure S3

Click here to download Supplemental Figure
s_figure4_160125.pdf

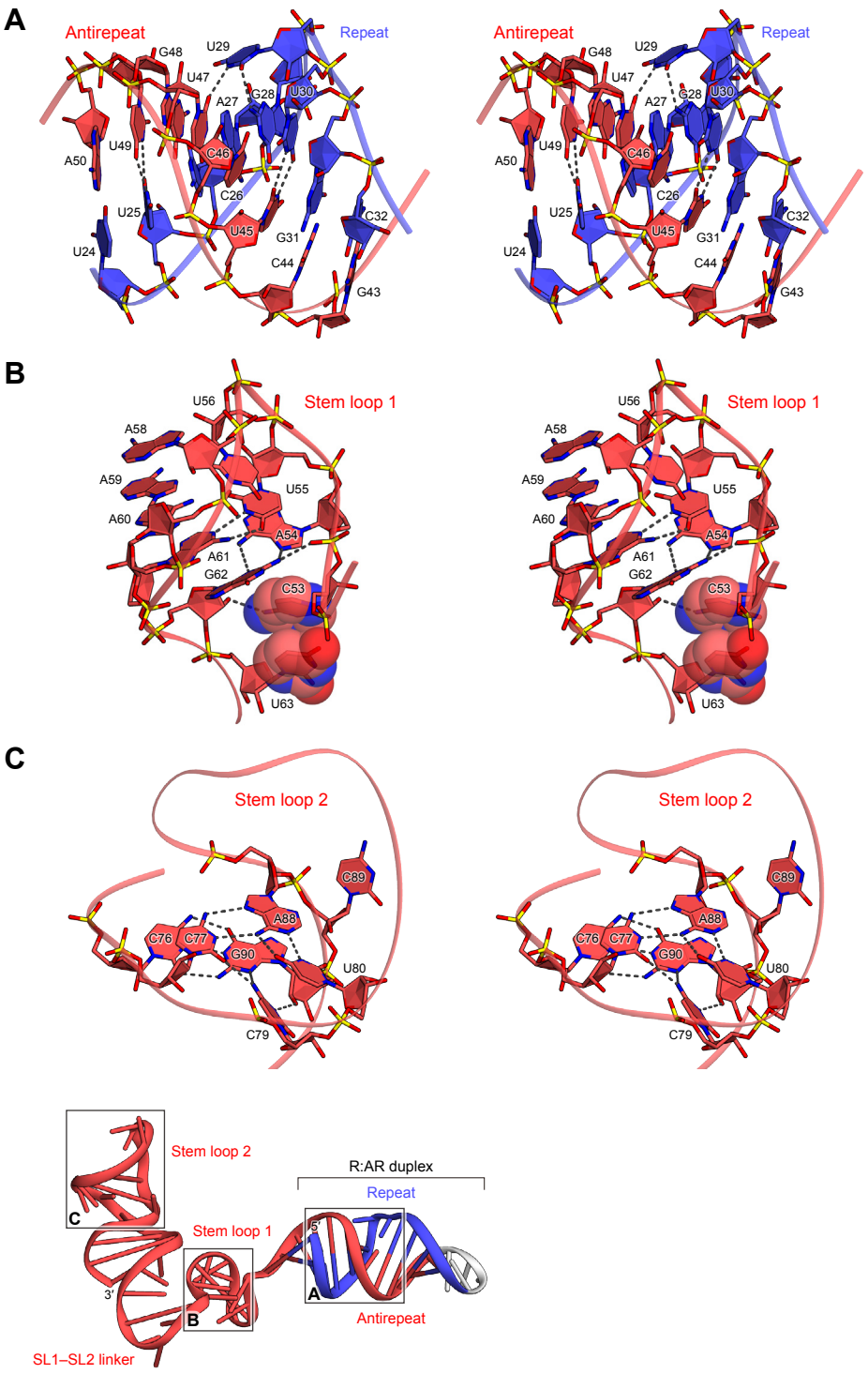


Figure S4

Click here to download Supplemental Figure
s_figure5_160125.pdf

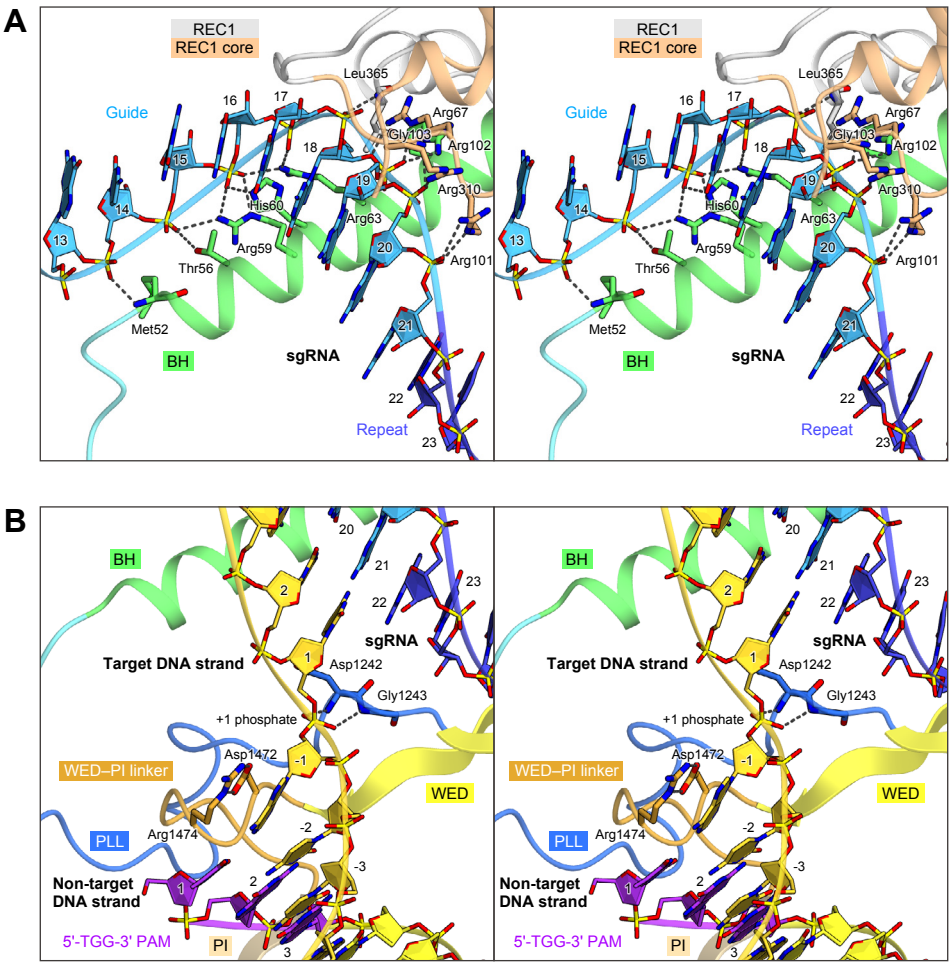


Figure S5

Click here to download Supplemental Figure
s_figure6_160125.pdf

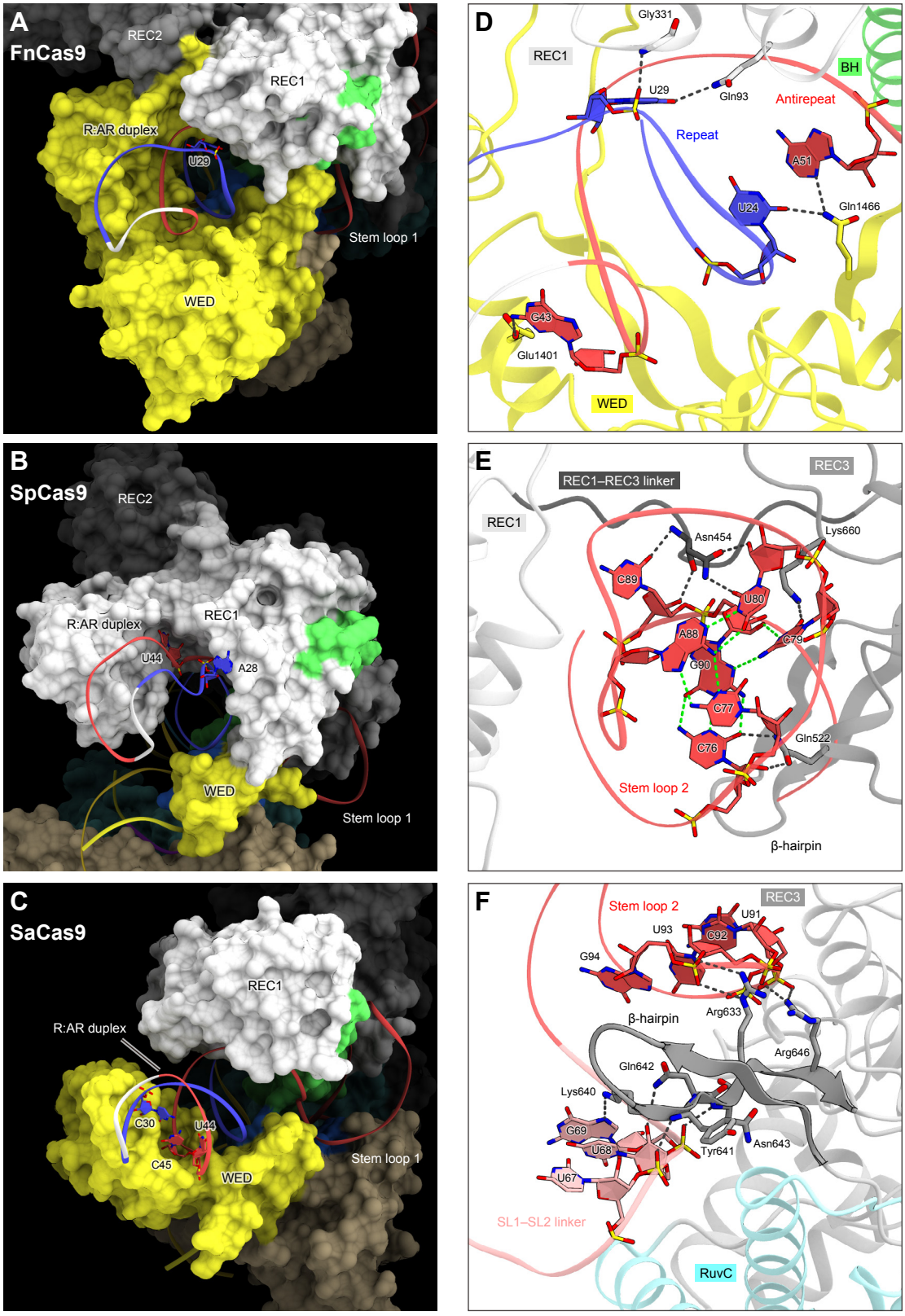


Figure S6

Click here to download Supplemental Figure s_figure7_160125.pdf

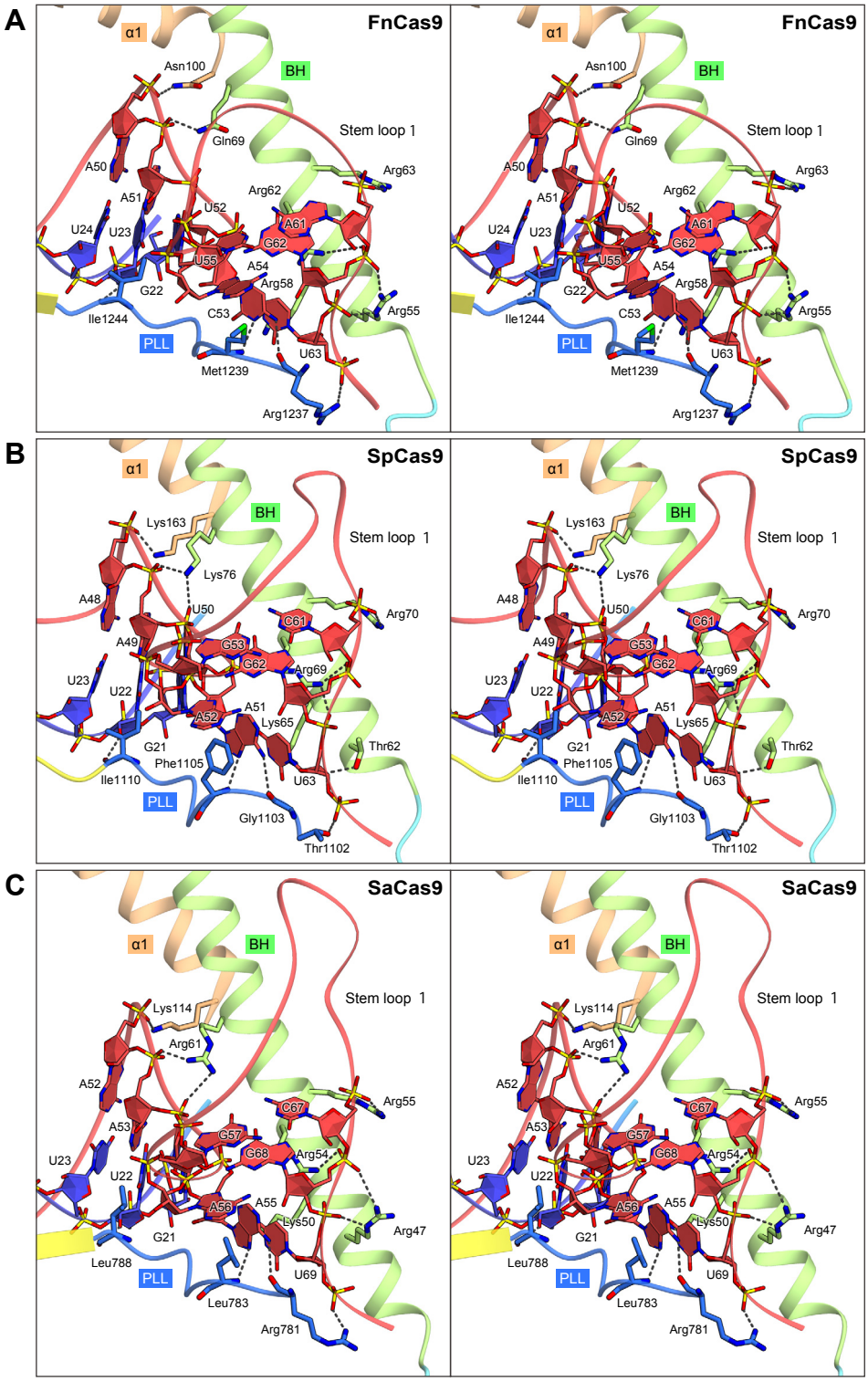


Figure S7